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ELECTROPHORESIS OF ISOENZYMES OF 16 WESTERN SHRUBS: TECHNIQUE DEVELOPMENT

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Abstract.— Wildland shrubs have gained considerable attention in recent years due to increasing recognition of their values as animal feed, as wildlife habitat, and for land reclamation. Better management of the shrub resource will be possible through clearer taxonomic identification and better understanding of phylogenetic relationships. This study applied polyacrylamide gel electrophoresis and further developed this technique to address genetic relationships among 16 paired shrub species (genera: *Artemisia, Chrysothamnus, Atriplex, Ceratoides, Sarcobatus, Purshia, Cowania,* and *Cercocarpus* [Compositae, Chenopodiaceae, Rosaceae]). Cluster analysis of similarity values for total protein and 14 isoenzyme systems gave patterns of species relationships expected from classical morphological grounds with two minor exceptions. Isoenzyme analyses showed promise for solving taxonomic, phylogenetic, and population genetics problems.

Wildland shrubs are receiving increasing attention as their value for land reclamation, wildlife habitat, livestock feed, and other purposes are better realized (McKell 1975). Several lines of research activity are currently underway to more effectively use shrubs as a resource to improve the nutrient quality of rangeland (Welch and McArthur 1979), to reclaim mine spoils (Thames 1977), and to maintain habitat diversity for productive rangelands (Plummer et al. 1968). As the shrubs receive more attention, their taxonomic identities and relationships need clarification so they can be better managed. Work is progressing in this taxonomic clarification effort. Examples are: saltbushes (*Atriplex*), using cytogenetics (Stutz et al. 1979); rabbitbrushes (*Chrysothamnus*), using paper chromatography (McArthur et al. 1978) and morphological characteristics (Anderson 1980); and sagebrushes (*Artemisia*) using paper chromatography (Hanks et al. 1973, West et al. 1978); morphological characteristics (Winward and Tisdale 1977, McArthur and Welch, in press); and cytogenetics (McArthur et al. 1981). This study was designed to further the taxonomic clarification effort by developing a polyacrylamide gel electrophoretic technique applicable to wildland shrubs. Sixteen plant species in three major western shrub families (Rosaceae, Chenopodiaceae, Compositae) were chosen to develop and test the technique.

Electrophoresis as a technique has been demonstrated to be of value in the analysis of isoenzymes of many plant and animal species (Gottlieb 1971). Isoenzymes (isozymes) are different molecular forms of enzymes with the same catalytic activity that migrate differentially during the electrophoretic process

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(Brewer and Sing 1970). Pattern correlation of several isoenzyme systems in concert has been taken as evidence of genetic relationship (Gottlieb 1971, Crawford and Wilson 1979, Kato and Tokumasu 1979). Study of isoenzymes has been important in developmental as well as population genetic studies (Shannon 1968, Conkle 1972, Myers 1978, Hartl 1980). This study, however, is confined to technique application and development for the three shrub families. The plant relationships were evaluated by comparing the isoenzyme data with other taxonomic information.

Materials and Methods

Plant Materials

Sixteen species of shrubs in eight pairs were chosen to develop and evaluate the technique (Table 1). The species were paired within genera according to their similar characteristics and taxonomic placement. Based on classical taxonomy, the pairs were thought to represent various degrees of phylogenetic affinity. One-half (eight) of the species are composites, with four *Artemisia* and four *Chrysothamnus* species chosen. The remaining half were chenopod and rosaceous shrubs (four species of each). Within *Artemisia*, two species pairs (pairs one and two) were used. *Artemisia absinthium* (wormwood) and *A. frigida* (fringed sage) both belong to the subgenus *Artemisia*, whereas *A. tridentata* ssp. *tridentata* (basin big sagebrush) and *A. nova* (black sagebrush) are members of the subgenus *Tridentatae* (McArthur et al. 1979, 1981). Species pairs three and four were chosen from *Chrysothamnus*, viz. *C. parryi* ssp. *attenuatus* (Parry rabbitbrush) and *C. nauseosus* ssp. *albicaulis* (white rubber rabbitbrush) from the section (subgenus) *Nauseosi*; and *C. viscidiflorus* ssp. *lanceolatus* (mountain low rabbitbrush) and *C. linifolius* (spreading rabbitbrush) from the section *Chrysothamnus* (Hall and Clements 1923, McArthur et al. 1978). Two pairs of cheno-

TABLE 1. Sources of plant materials used in isoenzyme study of 16 shrub species.

Family and taxa	Accession[1] and location of seed collection
Compositae	
Pair 1	
Artemisia absinthium (Arab)[2]	Field collection, N Provo, Utah County, Utah
Artemisia frigida (Arfr)	U-15, Sheep Creek, Sevier County, Utah
Pair 2	
Artemisia tridentata ssp. *tridentata* (Artr[t])	U-76, Clear Creek, Sevier County, Utah
Artemisia nova (Arno)	U-27, East Beaver bench, Beaver County, Utah
Pair 3	
Chrysothamnus parryi ssp. *attenuatus* (Chpa[a])	U-4, Ephraim Canyon, Sanpete County, Utah
Chrysothamnus nauseosus ssp. *albicaulis* (Chna[a])	North Hollow, Mayfield, Sanpete County, Utah
Pair 4	
Chrysothamnus viscidiflorus ssp. *lanceolatus* (Chvi[l])	U-13, Clear Creek Canyon, Sevier County, Utah
Chrysothamnus linifolius (Chli)	Field collection, Helper, Carbon County, Utah
Chenopodiaceae	
Pair 5	
Atriplex canescens (Atca)	U-103p, Rincon Blanco, Rio Arriba County, New Mexico
Atriplex lentiformis (Atle)	Field collection, 16 km S of Phoenix, Maricopa County, Arizona
Pair 6	
Sarcobatus vermiculatus (Save)	U-6, Ephraim, Sanpete County, Utah
Ceratoides lanata (Cela)	U-45, Diamond Mountain, Uintah County, Utah
Rosaceae	
Pair 7	
Purshia tridentata (Putr)	U-28, Mt. Pleasant, Sanpete County, Utah
Cowania mexicana ssp. *stansburiana* (Come[s])	U-20, American Fork, Utah County, Utah
Pair 8	
Cercocarpus montanus (Cemo)	U-28, Salina Canyon, Sevier County, Utah
Cercocarpus ledifolius (Cele)	U-42, East of Milford, Beaver County, Utah

[1]Taxa with "U" numbers assigned are plants from native accessions maintained at the Snow Field Station uniform garden in Ephraim, Utah.
[2]Taxa abbreviations adapted from Plummer et al. 1977.

pod shrubs were chosen. *Atriplex canescens* (fourwing saltbush) and *A. lentiformis* (big saltbush) constitute pair five. Both are included in a group of related woody dioecious shrubs by Hall and Clements (1923), and both have the evolutionary advanced C_4 mode of photosynthesis (Welkie and Caldwell 1970, Hatch et al. 1972), but they have distinctive floral and morphological differences (Brown 1956). The other chenopod pair (pair six), *Sarcobatus vermiculatus* (black greasewood) and *Ceratoides lanata* (winterfat), are probably the most loosely connected pair in the study. Both have C_3 photosynthesis (Welkie and Caldwell 1970), but are morphologically dissimilar. In fact, Standley (1916) placed greasewood closer to the saltbushes than to winterfat. The four rosaceous shrubs are all in the subfamily Rosoideae (Benson 1957). *Purshia tridentata* (antelope bitterbrush) and *Cowania mexicana* ssp. *stansburiana* (Stansbury cliffrose) comprise pair seven. Although placed in different genera, they are in fact closely related. Hybridization and introgression are common between the two species (Stutz and Thomas 1964, Blauer et al. 1975). The last pair (pair eight) was *Cercocarpus montanus* (true mountain mahogany) and *C. ledifolius* (curlleaf mountain mahogany). This species pair is related closely enough to produce a number of interspecific hybrids (Blauer et al. 1975).

Seeds were obtained from native accessions and from transplanted shrubs maintained at the Snow Field Station at Ephraim, Utah (Table 1). Seedlings were germinated under greenhouse conditions in fine-textured sand within 15 cm plastic pots and kept moist throughout germination. Commercial fertilizer consisting of a basic nitrogen-potassium-phosphorus (20:20:20) composition (no micronutrients) was applied weekly, in solution, to seedlings following emergence. It was found that growing seedlings in sand permitted better drainage and aeration, with fewer problems with fungus and other pathogens than was evidenced with soil as a growth medium. Temperatures during growth ranged from approximately 18 to 25 C.

Plant material was used from three general stages of growth: seedling (to approximately 20 pairs of leaves), juvenile (from seedling stage to young plants starting to become woody, usually less than 30 cm in height), and adult plants (fully grown, from field or garden collection sites). Plants were consistently used from accessions listed in Table 1, to reduce intraspecific variation (one accession per species). Leaf tissue was used for all extractions, with care to exclude stem or petiole, dehydrated portions of leaves, and extraneous matter.

Experimental Methods

To estimate approximate protein loadings of the samples used in electrophoresis, protein assays were conducted in accordance with Bio-Rad Laboratories[1] technique (Bio-Rad 1979). Due to the small sample size used in the study (approximately ½ ml) adjustment of the protein concentration within samples was not practical. Both dry weight and protein determination were conducted on samples at each growth stage. Vertical polyacrylamide gel was selected for the investigation because of the availability of commercial preformed gradient gels, superi or resolving ability, pore size range, and less fragile texture. Slab gels used also permitted better comparison of banding between samples than conventional tubular (disc) gels (Leaback 1976). Pharmacia polyacrylamide gradient gels (PAA 4/30) were used. These gels have a maximum polyacrylamide concentration of from 50,000 to 2 million daltons (Pharmacia 1978).

One hundred milligrams of fresh leaf tissue was carefuly weighed and placed in small prechilled mortars in a tray of cracked ice. To each leaf sample, ¼ ml refrigerated (2 C) Tris-boric buffer solution, pH 8.4, was added (Pharmacia n.d.), composition 0.09 M Tris, 0.08 M boric acid, 0.93 g/l Na_2EDTA. Density was increased by adding ¼ ml of a 40 percent sucrose solution to prevent diffusion of the sample into the reservoir buffer (Kuhns and Fretz 1978). To protect the proteins and

[1]The use of trade, firm, or corporation names in this publication is for information and convenience of the reader. Such does not constitute an official endorsement or approval by the U.S. Department of Agriculture or Brigham Young University of any product or service to the exclusion of others that may be suitable.

prevent formation of oxidation products, 3 μl of 2-mercaptoethanol (Thioglycol), 2 mg of L-ascorbic acid, and 2 mg of polyvinylpyrrolidone (PVP) were added to each sample.

In developing electrophoresis and staining procedures suitable for application to western shrub taxa, several problems were encountered. One of these was caused by the high levels of monoterpenoids and phenolics found in several species selected (Kelley and Adams 1977, McArthur et al. 1979, and Welch and McArthur 1981). Phenolics can form complexes with proteins and become readily oxidized. To lessen the probability of this interaction, PVP was added to extractions with 2-mercaptoethanol and ascorbic acid to bind phenols, protect protein integrity, and reduce smearing (Montgomery and Sgarbieri 1975). An additional problem was encountered in keeping the electrophoresis buffer cold to prevent inactivation of enzymes, with resulting pattern distortion (Brewer and Sing 1970). A pumping system was improvised that circulated ice water from a sink through cooling coils surrounding the buffer vessel, maintaining the temperature within prescribed limits (<10 C). The fresh leaf tissue was ground with additives in a small mortar and pestel (after Brewer and Sing 1970) until the tissue was well macerated and the mixture appeared homogeneous. This mixture was then centrifuged at approximately 10,000 g for about 10 minutes. Supernatant was withdrawn with pasteur pipette and analyzed by electrophoresis.

The electrophoresis apparatus used throughout this study was a Pharmacia vertical gel electrophoresis apparatus, GE-4 II, with accompanying power supply EPS 400/500 and accessory items (Pharmacia 1978). The supernatant was loaded into wells of an equilibrated slab gel with a 10-μl microsyringe. Gradient 4/30 gels (4 to 30 percent polyacrylamide concentration) were used for the study, due to having higher resolving power than conventional nongradient gels.

The gel was charged at 70 volts (v.) constant voltage for 20 minutes until the samples began to migrate into the gel. Electrophoresis was then carried out for 15 hours at 150 v. (constant voltage) with the surrounding buffer temperature maintained between 5 and 10 C. Following electrophoresis, the gels were stained either for total protein or for particular enzyme activity. Total protein staining procedure consisted of exposing gels to a 10 percent sulfosalicylic acid solution for 30 minutes, followed by approximately 90 minutes in a solution of Aniline Blue-Black (acid black 1), in 25 percent methanol, 10 percent acetic acid, and water (v,v,v). Destaining was done using the Pharmacia destainer GD-4 II with a solution of 25 percent methanol, 10 percent acetic acid, and water (v,v,v) for approximately 60 to 90 minutes until background stain was removed. To detect isoenzyme bands, a series of 14 enzyme recipes were used (Scandalios 1969, Shaw and Prasad 1970), with modifications listed in Table 2. Scandalios listed references designed for 10 percent acrylamide gels, necessitating changes in incubation periods to accommodate the 4–30 percent Pharmacia gels. Selection of enzyme stains was based on a review of success achieved in previous studies, availability of ingredients, and simplicity of technique.

Bovine serum albumin (BSA) was found to produce consistent banding patterns between runs when stained with total protein preparation and was subsequently used as a reference to operating conditions. Six bands resulted with BSA which, in 24 replications, produced standard mean errors (SE) of less than 0.4 mm. Because of this consistency between runs the migration distances are expressed in terms of millimeters of migration from origin. The BSA band, which migrated to approximately 59 mm, was densely stained and used for reference for migration rates for all isoenzyme and protein bands.

All banding patterns were scored on a light table for position and intensity. Distance to each band center from the top surface of the gel was noted, and bands were assigned a relative intensity value on a range from 1 (faint band) to 5 (darkly stained). When negatively stained bands were present, positions were recorded as for other bands and an intensity of "1" assigned. Data were compiled, using the 13 effective enzyme-staining recipes plus total protein, for each of the selected species at seedling stage (glutamic oxytransaminase

[GOT] was not effective). In addition, several enzyme stains (4 for juvenile plants and 5 for adult plants) plus total protein were applied to more mature plants so that pattern differences between growth stages could be compared. A minimum of three runs were made on each stage and plant species, and results compiled to produce an overall pattern of reproducible bands (Table 3). Banding data were clustered for percentage of similarity (s) between species, using the following formula:

$$s = \frac{C-.1x}{T}$$

where C represents the number of bands in common between taxa compared;
T represents the total of different bands present in either taxa, combined;
and x represents the absolute value of the sum of intensity differences of taxa.

A weighting factor of 10 percent (.1) was deducted from the similar bands with differences in band intensity. This method of comparison has been used by Whitney et al. (1968) and McArthur et al. (1978), but without a weighting factor. Whitney et al. suggest, however, that band intensity may have taxonomic significance.

TABLE 2. Enzyme banding characteristics used in evaluating 16 shrub species.

Enzyme	Pattern features	Taxa resolution value and enzyme consistency	Original source	Modifications
Peroxidase	Blurred banding	Fair/good	Shaw & Prasad 1970	Refrigeration time increased to 3 hours
Amylases	Faint; one band/taxa	Fair/good	Scandalios 1969	None
Esterases	One band/taxa	Fair/good	Scandalios 1969	Incubation time increased to 3 hours
Catalase	One band/taxa	Fair/good	Scandalios 1969	H_2O_2 concentrated increased to 10 percent; incubation time increased to 3 hours
Alcohol dehydrogenase (ADH)	Negatively stained bands present	Good/good	Scandalios 1969	Incubation time increased to 3 hours
Malate dehydrogenase (MDH)	Negatively stained bands present	Fair/good	Scandalios 1969	Stained in dark
Glutamate dehydrogenase (GDH)	Stained bands present	Poor/good	Shaw & Prasad 1970 Mitton et al. 1979	Stained in dark
Glucose-6-PO_4 dehydrogenase (G-6-PD)	Many bands (or none) present/taxa	Poor/fair	Shaw & Prasad 1970	Stained in dark
Acid phosphatase	One band/taxa	Fair/good	Scandalios 1969	Incubation time increased to 16 hours
Alkaline phosphatase	Two color bands present	Fair/good	Scandalios 1969	None
Leucene amino-peptidase (LAP)	Two color bands present	Fair/good	Scandalios 1969	Incubation time increased to 4 hours
Phosphoglucose isomerase (PGI)	Often blurred banding	Good/fair	Shaw & Prasad 1970	Stained in dark
Phosphoglucomutase (PGM)	Negatively stained bands present	Fair/fair	Shaw & Prasad 1970 Mitton et al. 1979	Stained in dark
Glutamic oxytransaminase (GOT)	Blurred; no recognizable banding characteristics	Poor/poor	Shaw & Prasad 1970	Used Fast Blue BB salt

TABLE 3. Isoenzyme positions and intensity for the 16 shrub species at the seedling stage.

Enzyme	Species and isoenzyme migration distance from origin (mm)[1]							
	Arab	Arfr	Artr[4]	Arno	Chna[a]	Chpa[a]	Chli	Chvi
Peroxidase	49/2 55/3 62/3 65/5	49/2 55/2 62/2 65/3	55/2 62/1	52/2 62/1 65/3	52/4 54/3 58/1	52/4 54/3	55/2 62/1	52/1 54/1 58/3 60/4
GDH	24/3	24/3	24/3	24/3	24/3	24/3	24/3	24/3
MDH	23/1	23/1	23/1	23/1	23/1	22/1	24/1	22/1
PGI	23/1 30/1 38/2 47/3	23/1 30/1 38/2 47/3	23/1 30/1 38/2 47/3	23/1 30/1 47/3	20/1 24/3 32/1 48/3 52/3	20/1 24/1 32/1 48/3 52/3	20/1 24/1 32/1 48/3 52/3	20/1 24/1 32/1 48/3 52/3
Esterase	24/3	24/3	24/3	24/3	24/3	24/3	24/3	24/3
Catalase	21/3	21/3	21/3	21/3	21/3	21/3	21/3	21/3
ADH	23/3 69/1	23/3 74/1	23/3 50/1	23/3 50/1	22/3 50/1 72/1	22/3 50/1 60/1 70/1	22/3 67/1 70/1	22/3 53/1 67/1 70/1
LAP	23/3 51/3	23/3 51/3	23/3 53/3	23/3 53/3	23/3 51/3	23/3 51/3	23/3 51/3	23/3 51/3
Amylases	22/2	22/2	22/2	22/2	22/2	22/2	22/2	22/2
Alkaline Phosphatase	23/3 55/2	23/3 46/1 51/1	23/3 46/3 51/1	23/3 46/2	23/3	23/3	23/3 46/1	23/3 46/1
G-6-PD	9/1 12/1 17/1	7/1 9/1 13/1 18/2	7/1 8/1 10/2 13/1	6/1 7/1 9/2 13/2 18/2	8/2 9/2 12/2 14/2 20/1	8/2 9/2 12/2 14/2 20/1	8/1 9/2 13/2 20/2	8/2 9/2 11/2 14/2 20/1
PGN	23/2 47/3 63/4	23/2 47/3 63/4	24/2 47/3 63/2	24/2 47/3 63/2	25/2 31/2 48/3 63/2	25/2 31/2 48/3 63/2	25/2 31/2 48/3 63/2	25/2 31/2 48/3 63/2
Acid Phosphatase	24/3	24/3	24/3	24/3	25/3	23/3	23/3	25/3

[1]Numbers expressed represent the migration distance in millimeters, followed by the intensity for each band; symbol designation for taxa are listed in Table 1.

Clustering of *s* values (Table 4) representing characteristic bands appears in Figure 1. The dendrogram in Figure 1 was constructed using a cluster analysis technique (McArthur et al. 1978). Stems of the dendrogram were tied together in descending order according to *s* value for all taxa being united above each union. Figure 2 illustrates patterns produced by seedling stage plant material when stained for specific enzyme activity.

RESULTS

Banding positions indicated in Tables 3 and 5 illustrate and confirm the more similar relationships anticipated within pairs of species and within genera than within families, and greater similarity of species within families than between family groupings. Figure 1 illustrates the relationships found between taxa. Of the enzyme stains used during the study for taxa differentiation, several were superior, namely, alcohol dehydrogenase (ADH), phosphoglucose isomerase (PGI), and phosphoglucomutase (PGM), in the number of bands present, resolution value, and consistency (Table 2). Others were also productive but offered less distinction between taxa.

Variability in banding patterns may be representative of age (Whitney et al. 1968) or environmental variation (Pandey 1967, Latner and Skillen 1968, Myers 1978). Differences in banding were noted between stages of growth, but not in a predictable manner. Clusters assembled from enzyme banding patterns at later growth stages were similar to those found within seedling stage plants in most cases. Juvenile stage shrubs clustered consistently within pairs, genera, and families. Adult plants did so, too, except for separation at the genus level of two *Artemisia* species and at the family level of the *Atriplex* species. In both more mature stages, the level of clustering between taxa was generally lower. Much of this difference may have

Table 3 continued.

Enzyme	Species and isoenzyme migration distance from origin (mm)[1]							
	Atca	Atle	Save	Cela	Putr	Come[s]	Cemo	Cele
Peroxidase	32/2	37/2	44/1 54/1	54/1	47/1	38/1 44/1 52/2	44/1 55/1	–
GDH	–	–	22/1	22/1	22/1	22/1	22/1	22/1
MDH	25/1	25/1	20/1	22/1 37/1	23/1	23/1	23/1	23/1
PGI	51/5 53/2 55/2	51/5 53/2 55/2	51/2	45/3 51/2	45/1 49/1	45/1 49/1	45/1 49/1	45/1 49/1
Esterases	–	–	–	–	22/2	–	–	–
Catalase	–	23/1	20/3	23/1	23/3	23/3	23/3	23/3
ADH	23/1 61/1	23/1 37/1 56/1 72/1	21/3 58/1	–	23/1	23/1	23/1	–
LAP	21/3 55/2	21/3 55/2	22/1 55/1	19/1 55/1	21/1 55/2	21/1 55/2	21/1 55/2	21/1 55/2
Amylases	–	25/1	22/2	–	23/1	23/1	23/1	–
Alkaline Phosphatase	–	24/2 50/1	21/2 50/1	–	23/2	23/2	23/2	23/2
G-6-PD	–	–	–	–	–	–	–	–
PGC	58/1 52/3 63/3	48/3 52/3 63/3	38/1	45/2 49/2 63/1	44/1 49/3 53/2	48/3	42/1 51/3	42/1 51/3
Acid Phosphatase	27/1	27/1	–	–	24/2	24/2	24/2	24/2

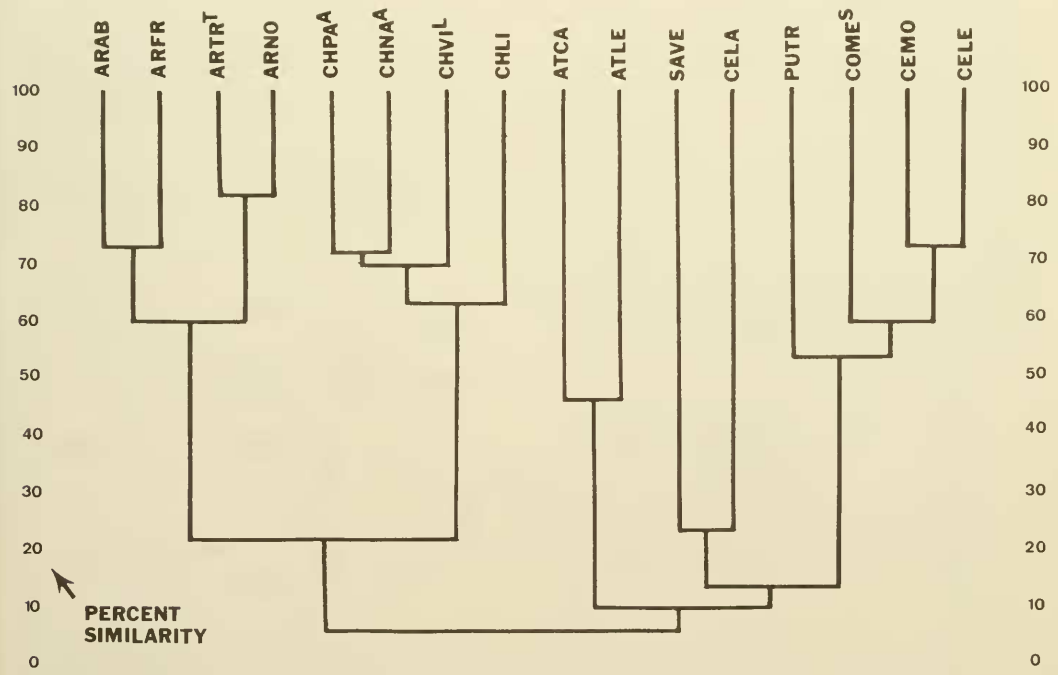

Fig. 1. Dendrogram of 16 shrub species based on similarity (*s*) value. (Symbol designations for taxa are listed in Table 1.)

been due to fewer enzyme systems used in their analysis.

Percentage dry weight of fresh leaf tissue ranged from approximately 30 to 80 percent, depending on taxa and stage of growth. Morrison (1961) stated that winterfat (*Ceratoides lanata*) possesses approximately 65 percent total dry matter, sagebrush leaves approximately 50 percent dry matter, and saltbush 28 percent dry matter. This study confirmed these findings, with adult basin big sagebrush and black sagebrush averaging 45 percent dry weight; big saltbush 35 percent, and winterfat 63 percent.

Protein concentrations using the Bio-Rad technique varied between approximately 1.1 mg/ml and 2.0 mg/ml at the seedling stage (depending on taxa). Several of the selected taxa displayed increases in protein levels upon maturation.

Discussion and Conclusions

The cluster analysis of seedling materials showed similarities between all but two of the proposed species pairs (Table 1, Fig. 1). The two exceptions were the mountain low rabbitbrush-spreading rabbitbrush and the bitterbrush-cliffrose pairs. In the rabbitbrush case, the two species have an *s* value of 66 percent (Table 4), but the low rabbitbrush clusters slightly closer ($s = 70$) to the two *Nauseosi* species. Spreading rabbitbrush has an *s* value (66) more similar to that of low rabbitbrush than do the other two rabbitbrush species ($s = 58$ and 65). All the rabbitbrushes together cluster at a high level ($s = 67$). The other exception (bitterbrush-cliffrose) to the proposed pairs occurs because cliffrose clusters more tightly with true mountain mahogany ($s = 67$) than it does to bitterbrush ($s = 55$). The cliffrose *s* value with curlleaf mountain mahogany is 53. Although cliffrose is considered to be allied closer to bitterbrush than to the mahoganies (Stutz and Thomas 1964), it shares with the mahoganies the floral characteristic that bitterbrush lacks, namely, persistent plumed styles (Blauer et al. 1975).

The *Artemisia* pairs cluster in each subgenus at *s* values > 70. *Artemisia* and *Chrysothamnus* are in different composite tribes, *Anthemideae* and *Astereae*, respectively. Our cluster diagram (Fig. 1) supports their within-family relationship.

The chenopod pairs both clustered as anticipated. The saltbush pair has an *s* value of 46 (Table 4, Fig. 1). These saltbushes have affinities in photosynthetic pathways and shrubby habit (Welkie and Caldwell 1970), but contrasts in floral, fruit, and leaf characteristics (Hall and Clements 1923, Brown 1956). Greasewood and winterfat have the lowest *s* value (17) of all pairs. This low value is consistent with Standley's (1916) placement of them in separate subfamilies. Both were distant enough from the saltbushes that they clustered more closely with the rose family shrubs. We found it interesting that there was some isoenzyme similarity across

TABLE 4. Percent similarity of isoenzyme bands for 16 shrub species—seedling stage.

	Taxa[1]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Arab															
2	Arfr	73														
3	Artr[4]	49	64													
4	Arno	52	72	83												
5	Chpa[a]	23	18	19	19											
6	Chna[a]	24	21	22	22	72										
7	Chvi[1]	17	20	17	18	68	71									
8	Chli	21	27	27	25	65	58	66								
9	Atca	4	4	4	4	2	2	2	2							
10	Atle	4	4	4	4	4	6	4	4	46						
11	Save	2	2	2	2	4	5	4	5	11	9					
12	Cela	2	2	2	2	7	5	7	5	10	8	17				
13	Putr	9	9	9	9	2	4	2	2	3	9	7	18			
14	Come[5]	10	10	10	10	6	10	6	5	11	13	11	15	55		
15	Cemo	12	12	12	12	2	4	2	2	11	9	11	15	55	67	
16	Cele	8	8	8	8	2	5	2	2	8	6	8	18	50	53	73

[1]Symbol designations for taxa are listed in Table 1.

all 16 of these diverse shrub species (Fig. 1, Table 4).

In an earlier study (McArthur et al. 1978), comparisons of similarity among some of the same *Chrysothamnus* taxa were reported by comparing phenolic compounds. With isoenzymes (this study), the overall rabbitbrush average *s* value was 67 compared to 42 with

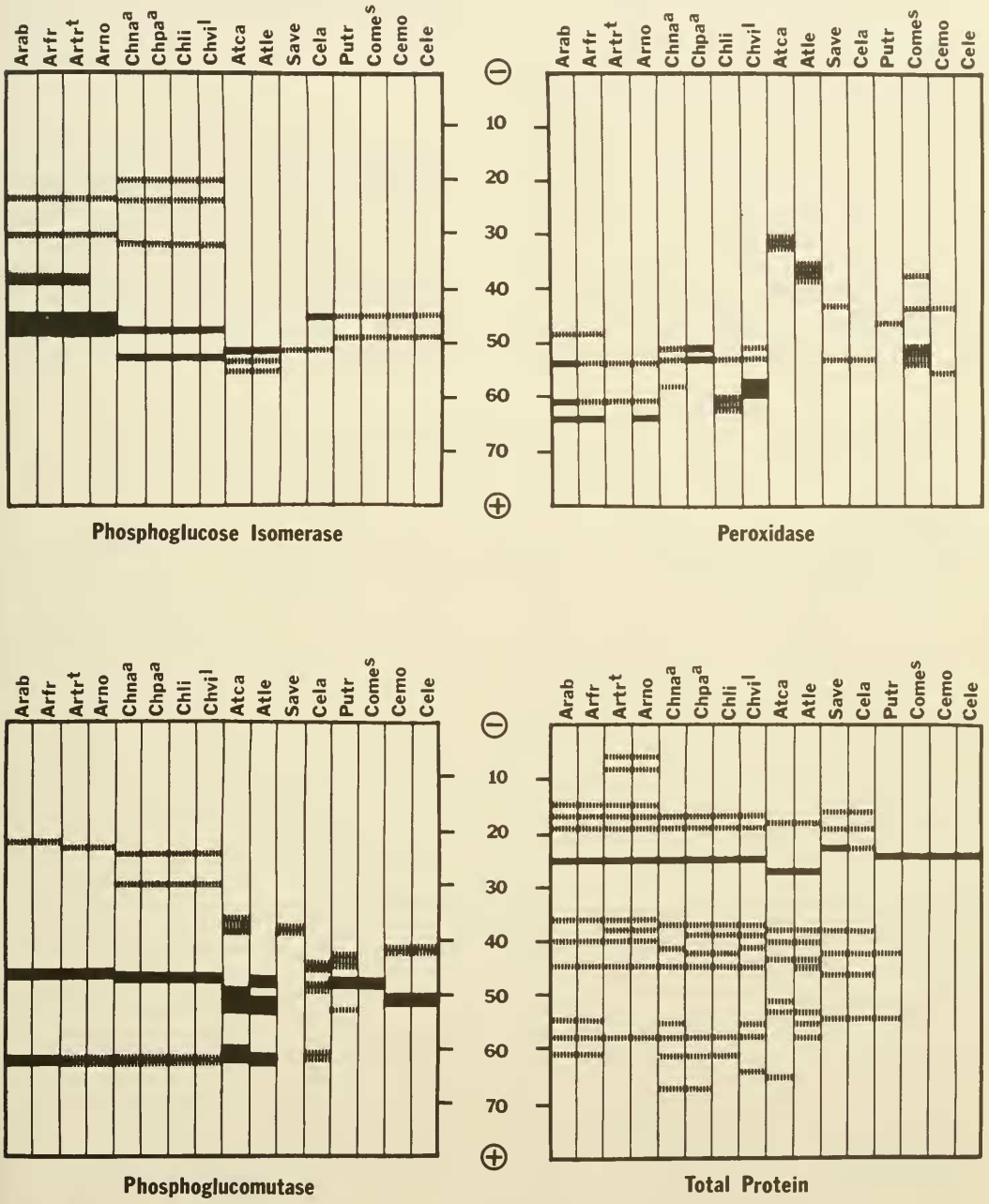

Fig. 2. Zymograms of selected isoenzymes and total protein for 16 shrub species. (Symbol designations for taxa are listed in Table 1.)

the phenolic compounds. It may be significant that isoenzymes are biosynthetically closer to their DNA encoders than are phenolics (Wagner and Mitchell 1964).

Polyacrylamide gel electrophoresis offers several distinct advantages as a technique for investigating genetic or evolutionary relationships among taxa of interest. The preformed gradient gels eliminate the time of preparation and exposure to toxic unpolymerized acrylamide. Gradient concentration with corresponding variations in pore size permits a sieving effect that tends to sharpen and accentuate differences in isoenzyme mobility (Brewer and Sing 1970, Leaback 1976). This separation assists in characterizing closely related taxa. The results of this study using morphologically distinct taxa will permit further studies on narrower, taxonomically difficult shrub groups. The results also point out the opportunity for studies designed to gain better understanding of the breeding systems and population genetics of *Artemisia, Chrysothamnus, Atriplex, Ceratoides, Sarcobatus, Purshia, Cowania,* and *Cercocarpus.*

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TABLE 5. Protein band positions within 16 shrub species[1]—seedling stage.

	Species and migration						
Bovine serum albumin 1.0% solution[2]	Arab	Arfr	Artr[4]	Arno	Chna[a]	Chpa[a]	Chli
			6	6			
			8	8			
	15	15	15	15			
	17	17	17	17	17	17	17
23.2 ± 0.3 n=24							
	25	25	25	25	25	25	25
28.5 ± 0.3 n=24							
34.4 ± 0.3 n=24							
	36	36	36	36			
					37	37	37
			38	38			
						39	39
	40	40	40	40			
					41	41	
							42
44.0 ± 0.4 n=26							
	45	45	45	45	45	45	45
	55	55			55		
58.8 ± 0.4 n=26	58	58	58	58	58	58	58
	61	61			61	61	61
66.7 ± 0.4 n=25							
					67	67	

[1]Symbol designations for taxa are listed in Table 1.
[2]A 1.0 percent solution of bovine serum albumin produced 6 characteristic bands with position, standard mean of the error, and sample size (n) as shown. These bands were used as a reference for consistency.

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Table 5 continued.

distances from origin (mm)								
Chvi[1]	Atca	Atle	Save	Cela	Putr	Come[5]	Cemo	Cele
			16	16				
17								
	18	18						
			19	19				
			23	23				
					24	24	24	24
25								
	27	27						
37								
	38	38	38	38				
39								
	40	40						
42			42	42	42			
	43	43						
45		45						
			46	46				
	51							
	53	53						
			54	54	54			
55		55						
58		58						
64								
	65							

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